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GT biplot analysis of shoot traits indicating drought tolerance in cowpea [*Vigna unguiculata* (L.) Walp] accessions at vegetative stage

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ABSTRACT
The present study was done to analyse trait association and genetic variation in cowpea under drought stress at vegetative stage by GT biplot analysis. Ten accessions of cowpea were grown in pots filled with 7 kg of top soil in 3 replicates in completely randomised design (CRD). Each pot was applied 500 ml of water every other day for 5 weeks; then drought was imposed for 3 weeks by withholding watering. Data were collected on wilting parameters, stomata conductance and morphological characters. Plants were re-irrigated after 3 weeks of stress for another 2 weeks; collection of data on recovery parameters and number of pods were done. Data were subjected to analysis of variance and GT biplot analysis. Biplot revealed that wilting parameters excluding leaf wilting index, recovery traits and number of pods were very important traits to be considered for breeding for drought tolerance in cowpea. The highly susceptible accessions were AC08, AC10 and AC03, while the most tolerant accessions were AC06 and AC07.

Key words: GT biplot, drought tolerance, cowpea, wilting index, recovery

INTRODUCTION
Cowpea plays a significant role in the protein requirements of humans and animals making it one of the most important legume crop in the sub-Saharan Africa (SSA) and most especially in Nigeria. Small scale farmers depend on the crop for its nutrition potential, enhancement of soil fertility and income drive [1], [2], [3]. Cowpea grains are rich in calcium (826 mg/kg), magnesium (1915 mg/kg), phosphorus (5055 mg/kg), potassium (14,890 mg/kg), protein (25% content), iron (53.20 mg/kg) and zinc (38.10 mg/kg). It has the capacity to fix about 70 to 350 kg of nitrogen per hectare of soil making its projected potential impact between the periods of 2011 to 2020 lies around 77,320 tons [4]. Africa accounts for 83% of the worldwide cowpea production (6.5 MT/annum), more than 80% of Africa's production come from West Africa with 55% accounted to Nigeria being the world's largest producer at 45% estimated global production [1].

Despite the efforts of many workers at improving cowpea productivity against any known constraint, its productivity is hampered significantly by drought stress especially if exposed to drought at seedling stage, vegetative stage or terminal drought [3], [5]. Drought is one of the misfortunes associated with climate change as evident on plant growth and development, thereby propelling input of researchers in the direction of developing genotypes with

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enhanced adaptation strategies [4]. Molecular as well as conventional breeding methods have been adopted in developing improved lines of cowpea capable of withstanding drought stress in a bid to lessen the devastations caused by drought to the crop. Through screening of germplasm of different origins, sources of genes conferring drought tolerance have been identified. The abundant genetic resources of cowpea maintained in International Institute of Tropical Agriculture (IITA) Gene Bank (above 2000 wild relatives and about 15,000 accessions) provide the materials for mining resistance to abiotic stress like drought in cowpea as confirmed by several authors [1]. Several breeding approaches in different combinations and modifications have been adopted in hybridization programmes involving these genetic resources in which promising individuals in segregating populations have been selected for drought related traits in crops.

Screening of cowpea for vegetative stage drought tolerance has been one of the strategies of cowpea selection. Mechanisms found to associate with survival at vegetative stage include high leaf water status, limited changes in water potential and little osmotic adjustment under drought stress. Cowpea has stomata that are very sensitive to deficit of moisture in soil and respond accordingly by partial closing before changes in leaf water potential; it has difficult to wilt leaves which change to vertical orientation and track the sun in a way that reduces capture of solar radiation [6]. Several phenotyping methods have been used to identify drought tolerant cowpea genotypes among which wilting characteristics of shoot traits play a significant role by reducing the complexities associated with evaluation for the traits in crops [7]. Unfortunately, information regarding wilting characteristics of shoot traits in cowpea at vegetative stage is limited [8]. Understanding the relationship among shoot wilting characters and morphological traits can help in interpreting the difficult mechanisms conferring drought tolerance in cowpea.

Considerations must also be made on the relevant statistical tools for screening for drought tolerance [9], [10]. Relationships among traits and genotype profiling under drought stress have been reported in several studies in most cases employing regression and correlation analyses [11], but the recently adopted genotype-by-trait (GT) biplot analysis, developed from the GGE biplot [12] is a powerful multivariate tool for analysing relationships among traits and for selection of superior genotypes under drought stress [13], [14]. GT biplot analysis is superior to the simple correlation techniques for its capacity to graphically explain the interrelationships among measured traits based on overall pattern of the data as opposed to correlation which only describes the relationship between two traits. GT biplot also provides visual comparison among genotypes based on multiple traits and can be utilized in comparing selection schemes for parental selection and cultivar evaluation [13].

Since the performance of cowpea under drought stress at reproductive stage can be projected from its performance at the vegetative stage using multiple traits aside yield, since wide acceptability of genotypes is mainly on the basis of many desirable traits and not only on its yield potential; it is important to exploit multiple traits in assessing cowpea genotypes under drought stress at vegetative stage for effective selection. GT biplot analysis has been used in many crop species for assessing variability under stress and under normal conditions and has been successful especially in rice accession [15], tomato hybrids [16], wheat [17], [18] and in maize genotypes [14], [19]. It has also been adopted in trait profiling of cowpea genotypes and other legumes [11],

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[13], [20], [21], [22], [23]. Unfortunately, this tool has not been used to analyse relationships among shoot wilting characters and morphological traits of cowpea under vegetative stage drought stress.

Taking cognizance of the need to provide comprehensive information on association of shoot traits conferring drought tolerance in cowpea at vegetative stage and genotype profiles; the present study was set up to analyse trait association and genetic variation in cowpea under drought stress at vegetative stage by GT biplot analysis.

MATERIALS AND METHODS

Plant materials

Ten accessions of cowpea of various origins previously screened at the seedling stage [3] were utilized in the present study. Accessions were received from the International Institute of Tropical Agriculture (IITA), Nigeria and maintained at the Plant Breeding Unit, Department of Plant Science and Biotechnology. These accessions included TVu-199, TVu-207, TVu-218, TVu-235, TVu-236, TVu-241, IT98K-205-8, IT98K-555-1, TVu-4886 and TVu-9256 and respectively coded as AC01, AC02, AC03, AC04, AC05, AC06, AC07, AC08, AC09 and AC10.

Drought stress treatment

The study was done between March and May, 2016 at the screen house of Plant Science and Biotechnology Department within Adekunle Ajasin University Campus, Akungba Akoko, Nigeria.

The screening procedure was highly modified from [3]. A total of five seeds were sown in plastic pots filled with 7 kg sieved sandy loam soil devoid of fertilizer. Seedlings were thinned to two plants per pot two weeks after emergence. There were five pots utilized per accession in three replicates adopting a completely randomised design (CRD), making a total of 150 pots and 300 plants. Each pot was watered with 500 ml of tap water per day for five weeks after which watering was stopped to impose water stress for three weeks (21 days). Each pot had three perforations underneath to prevent water logging within the pots. Re-watering of accessions commenced immediately after 21 days of stress and this was done for two weeks (14 days) to assess recovery rate of accessions.

Measurement of parameters

Traits such as wilting parameters (susceptibility to drought score, percentage of wilted plants, leaf wilting index), stomata conductance, and morphological parameters (plant height, width and length of terminal leaflet and stem girth), number of pods and recovery parameters (percentage recovery, stem regrowth and stem greenness) after re-watering were taken. Susceptibility score was done on 1 to 7 scale (between 1.0 and 3.9: susceptibility is low; between 4.0 and 5.9: susceptibility is moderate; between 6.0 and 7.0: susceptibility is high). Leaf wilting index was done as the ratio of leaves showing wilting signs or wilted to the total number of leaves per plant [7]. These measurements were done at the interval of seven days for twenty one days. Stomata conductance (engaging a leaf porometer, Model SC-1, Decagon Inc. and done between 11.30 am and 5.00 pm on the same leaflet selected for width and length measurements at day 7 only). Morphological traits were assessed till day 14 of drought stress, except for terminal leaflet width and length assessed till day 7 of stress. Stem greenness was done on 1 – 5 scale [1 = yellow (plant recovered) and 5 = plant fully green). Plant regrowth was scored based on 1 – 5 scale [1 = no regrowth/plant recovered; 3 = regrowth at auxillary buds and 5 = regrowth at apical buds [7].

Statistical analysis

Analysis of variance (ANOVA) was done with SPSS version 20. Means separation was done

at P ≤ 0.05 level of significance utilising DMR test. All parameters were subjected to biplot analysis with Palaeontological Statistics (PAST) version 4.01.

RESULTS AND DISCUSSION

Analysis of variance (ANOVA) showed substantial variation between accessions for all traits under vegetative drought stress, suggesting a reasonable degree of genetic differences among such accessions (Table 1). Related variations were observed for the seedling stage drought tolerance among the same accessions screened [3].

Results for the wilting parameters are presented in Table 2. Wilting in plants can be seen visually as a result of drought stress, enabling the monitoring of various wilting intensities on cowpea plants. Results from this study showed that the cowpea accessions reacted differently to the stress, and the response depended on time. Using the drought susceptibility score based on the level of wilting in plants, all accessions showed wilting within 7 days of drought stress in accordance with several authors' findings [9], [23], [24], [25], [26], [27] which reported wilting from drought stress in cowpea within the first seven days. This indicates that vulnerable accessions can be easily detected in conducting assessments during the first week of drought stress [7]. Meanwhile, this was slightly at variance with the outcome of seedling wilting where only four of the accessions (AC10, AC08, AC02 and AC01) showed wilting within seven days of stress [3]. At day 21 of drought stress, AC10 became the most susceptible. Also, the percentage of fully dead or wilted plants for each accession was also consistent with each accession's level of susceptibility, as stated earlier [3], [25]. Number of wilted plants under drought stress has been found to be effective for selection of drought tolerant cowpea lines [28]. Within 7 days of stress, AC10 had the highest leaf wilting index, meanwhile high leaf wilting indices within the first week of drought stress have been used to select drought susceptible genotypes of cowpea [7]. At the 21st day of drought stress, leaf wilting index was generally high among accessions.
Upon re-watering for two weeks, plants of some accessions recovered fully (Table 3), although some continued to die, including accessions AC10 and AC08 where plant mortality reached 100 percent and this result was consistent with the seedling stage [3]. Among accessions, those with slow wilting from the onset of drought and also with lower susceptibility score (consistently) and low percentage of wilted plants at the end of drought stress, such as AC05, AC09, AC07 and AC06 sustained high re-growth and stem greenness and had high recovery rates. Accessions with the highest susceptibility never recovered. Recovered accessions obviously had an intrinsic mechanism to slow down their moisture loss by reducing the transpiration rate, unlike those with high wilting, as reflected in their stomata conductance. Similar results have been achieved in cowpea [29], common bean [30] and soy [31].

Results for morphological parameters, number of pods and stomata conductance are presented in Tables 4 to 9. Significant genotypic differences were seen between accessions for all traits under vegetative stage drought stress. Length of terminal leaflet reduced in all accessions except in AC09 and AC05 (Table 4), width of terminal leaflet also decreased in all but in AC09 (with the highest reductions of 11.82% observed in AC03) (Table 5). These results are in accordance to the findings of [28] which attributed reduction in leaf area in cowpea under drought stress to one of the mechanisms plants adopt to avoid higher transpiration rate and limited surface to radiation. Height of plant increased in all accessions with the least increment (0.33%)

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observed in AC10; AC07, AC09 and AC03 were among the most affected, while AC01 was the least affected (Table 6). This result agrees with previous reports on cowpea [28], [32] evaluated for plant height under different levels of drought stress. In other crops like sorghum and maize, negative effects of drought have been reported on both morphological and physiological traits. Reduction in plant height under stress has been linked to limited cell division and enlargement [33], [34], [35], [36], [37]. Stem girth also reduced in all accessions (Table 7); severe reduction in stem girth under drought stress has been reported in cowpea [38], and this in conjunction with reduced leaf area have been attributed to the mechanism by which plants control water use in order to reduce injuries linked to drought stress [32], [39]. These assertions agree with the findings of the present work, in that the accession with highest reduction of stem girth was AC07, which had the least level of susceptibility and the lowest percentage of wilted plants coupled with the lowest stem girth as at the end of the stress period. Larger stem girth under drought condition has been linked to enhanced drought tolerance in cowpea since such genotypes may possess the capacity to better accumulate carbohydrate in stems, thereby contributing to its tolerance to drought stress [8]. In the present study, AC08 and AC02 had the highest stem girth, while AC08 was one of the most susceptible, AC02 was only a moderately tolerant accession in contrast to the findings of [8].

The most susceptible accessions did not produce pods (Table 8); while stomata conductance (Table 9) was generally high in most susceptible accessions with significant differences. Agbicodo [27] reported significant reduction in grain, fodder and total yields of cowpea, and opined that drought stress at early stages of flowering and pod formation imposed significant damage to plant functions and consequently total biomass yield. Reduction in yield under drought stress has been reported in cowpea [40], [41]. In this study, aside AC09, all accessions such AC10, AC06, AC04, AC08 and AC03 showing the highest levels of susceptibility at 21st day of drought stress did not produce any pods.

At day 21 of stress, PC1 to PC3 accounted for 90.49 percent of the total variation. Traits like percentage recovery, stem regrowth, stem greenness and number of pods had high loadings with positive contribution to the total variation in PC1, while drought scores and percentage of wilted plants were high negative contributors. All traits were high positive contributors in PC2 aside number of pods. Li *et al.* [42] and [3] reported that PCA was effective for selecting the best characters for drought tolerance in maize and cowpea respectively.

The GT biplots with polygon views displaying the data of the ten accessions are presented in Figure 1 (a – f). Trends of characters associations varied under different durations of drought stress due to genotype by environment interaction as displayed by the GT biplots in this study. The GT biplots captured 57.50% of the total variations at day 7 (Fig 1a), 74.00% at day 14 (Fig 1c) and 74.67% at day 21 (Fig 1e) in PCs 1 and 2. The first two PCs should explain above 60 percent of the data variability [43], therefore, the present results reflect that most of the variations for each duration of drought were explained by PCs 1 and 2. This result agrees with the findings of [13]. Similar trends were also observed among the same accessions subjected to drought screening at seedling stage [10]. In the present study, all traits were important at day 7 of stress except leaf wilting index, stem girth and stomata conductance, at day 14 and 21 of stress, all traits involved were very important except leaf wilting index. The right hand of the biplots was dominated by morphological traits, number of pods and

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recovery parameters at day 7, while the left side of the biplots was occupied by the wilting parameters, stomata conductance and stem girth. At days 14 and 21, recovery parameters occupied the right hand of the biplots while the morphological and wilting parameters occupied the left side. Recovery parameters, number of pods and wilting parameters were very consistent at every interval from day 7 to 21. Vertex accessions in the sectors of corresponding traits in the biplots are regarded as the best accessions for such traits, and these could be exploited in crossing programs as promising parents in the breeding of genotypes and populations outstanding in those traits [13], [22]. In the presently study, AC06 and AC07 were the vertex accessions for the drought tolerant traits sectors such as the recovery parameters and number of pods respectively. The vertex accessions for the wilting traits sector included AC03, AC08 and AC10 which were the most susceptible accessions. Accessions AC06 and AC07 were the least susceptible to the drought stress, other accessions belonging to this group included AC09, AC02, AC01 and AC05; these accessions had pods and high recovery rates. AC06 did not produce pods but maintained greenness and highest recovery rate. Conversely, AC07 had the highest number of pods with moderate percentage recovery. Susceptible accessions which recovered and regrew included AC03 and AC04, hence their positions on the biplots.

Trait vectors are the projections of the trait markers from the origin of the biplot which also signify the approximate values of their standard deviation. Correlation between any two traits is defined by the cosine of the angle between the trajectories of the traits, hence, vectors of two traits with acute angle ($< 90^0$) have positive correlation, those of obtuse angle ($>90^0$) are negatively correlated, while those of approximately right angle ($=90^0$) have no correlation [13]. Bi-plot at vegetative stage revealed that percentage of plants wilted, drought susceptibility score and leaf wilting index were highly correlated and regarded as susceptible traits, while traits like stem greenness, regrowth and percentage recovery were highly correlated and regarded as drought tolerant traits. Highly susceptible accessions (AC08 and AC10) were clearly separated by bi-plot. Similar results were reported by [10] and [32]. Bibi *et al.* [35] successfully applied Principal Component analysis and bi plots to select drought tolerant traits and genotypes of sorghum under seedling drought stress. At day 7 of stress, all morphological traits were positively correlated: stem girth was highly correlated to wilting traits, also stomata conductance and wilting parameters were highly correlated. These indicated that accessions with higher stem girth had plants with higher stomata conductance under drought stress and were more susceptible to drought. At day 14 of stress, plant height and stem girth were positively correlated with the wilting parameters indicating that accessions with higher plant height and stem girth were more susceptible to drought stress. At day 21, number of pods was negatively correlated with recovery parameters indicating that accessions which produced pods (AC09, AC07, AC01 and AC02) had lower recovery rate as opposed to accessions which did not produce pods as found in AC06 and the lowest pods (AC05).

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Table 1: Mean square values for all parameters of accessions of cowpea under drought stress at vegetative stage

TRAIT	ACCESSION (DF = 9)	ERROR (DF = 20)	CV (%)
DSS7	1.09**	0.53	26.38
DSS14	0.45**	0.32	9.70
DSS21	0.98**	0.22	7.36
PPW7 (%)	342.22**	13.33	32.22
PPW14 (%)	668.14**	56.67	18.21
PPW21 (%)	455.19**	46.67	8.23
LWI7	0.05**	0.04	50.00
LWI21	0.03**	0.02	14.43
SCND (mmol $m^{-2}s^{-1}$)	107320.29**	4650.23	29.74
PREC (%)	1556.87**	108.49	35.38
STG	13.02**	0.13	9.17
STR	4.67**	0.13	15.47
ITLL (cm)	3.22**	0.90	11.36
TLL7 (cm)	3.18**	1.16	13.33
ITLW (cm)	2.27**	0.47	16.09
TLW7 (cm)	1.85**	0.49	17.77
IPH (cm)	14.69**	10.15	12.98
PH7 (cm)	39.22**	30.05	20.31
PH14 (cm)	39.22**	30.05	20.31
ISG (mm)	0.25**	0.19	11.72
SG7 (mm)	0.16**	0.10	12.78
SG14 (mm)	0.16**	0.08	24.04
PDP (mm)	101.37**	2.00	38.22

DF: Degree of freedom; **: Significant at P ≤ 0.05; ns: Not significant; DSS7, DSS14, 21: Susceptibility to drought score at day 7, 14 and 21; PPW7, 14, 21: Percentage of plants wilted at day 7, 14 and 21; LWI7, 14, 21: Leaf wilting index at day 7, 14, 21; SCND: Stomata conductance; PREC: Percentage recovery; STG: Stem greenness; STR: Stem re-growth; ITLL: Initial terminal leaflet length; TLL7: Leaflet length at day 7; ITLW: Initial terminal leaflet width; TLW7: Leaflet width at day 7; ISG: Initial stem girth; SG7, 14: Stem girth at day 7 and 14; PDP: Number of pods per plant.

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Table 2: Susceptibility to drought score (DSS), percentage of plants wilted (PPW %) and leaf wilting index (LWI) of accessions of cowpea under drought stress at vegetative stage

ACCESSION	DSS7	DSS14	DSS21	PPW7	PPW14	PPW21
AC01	2.67±0.29^{a}	5.60±0.58^{ab}	5.74±0.69^{ab}	0.00±0.00^{a}	30.00±0.00^{ab}	76.67±3.33^{bc}
AC02	3.13±0.07^{ab}	5.87±0.18^{ab}	6.27±0.27^{bc}	26.67±3.33^{d}	43.33±8.82^{bc}	70.00±0.00^{ab}
AC03	2.00±0.61^{a}	6.07±0.18^{ab}	7.00±0.00^{c}	20.00±0.00^{c}	43.33±3.33^{bc}	100.00±0.00^{f}
AC04	2.33±0.41^{a}	5.80±0.40^{ab}	6.60±0.11^{bc}	10.00±0.00^{b}	56.67±6.67^{cd}	93.33±0.00^{def}
AC05	2.80±0.72^{a}	6.00±0.23^{ab}	6.27±0.18^{bc}	10.00±0.00^{b}	56.67±3.33^{cd}	83.33±6.67^{cd}
AC06	2.40±0.35^{a}	6.33±0.27^{b}	6.40±0.31^{bc}	0.00±0.00^{a}	60.00±0.00^{d}	86.67±3.33^{cde}
AC07	2.47±0.35^{a}	5.00±0.00^{a}	5.13±0.13^{a}	6.67±3.33^{ab}	20.00±0.00^{a}	60.00±5.77^{a}
AC08	3.00±0.35^{ab}	5.80±0.53^{ab}	6.73±0.17^{c}	6.67±3.33^{ab}	50.00±5.77^{cd}	96.67±3.33^{ef}
AC09	2.60±0.40^{ab}	5.57±0.19^{ab}	6.53±0.07^{bc}	3.33±3.33^{ab}	20.00±0.00^{a}	76.67±6.67^{bc}
AC10	4.20±0.31^{b}	6.27±0.29^{b}	7.00±0.00^{c}	30.00±0.00^{d}	33.33±3.33^{ab}	100.00±0.00^{f}

Table 2 cont'd.

ACCESSION	LWI7	LWI21
AC01	0.26±0.06^{a}	0.91±0.07^{a}
AC02	0.43±0.06^{ab}	0.96±0.02^{ab}
AC03	0.48±0.19^{ab}	1.00±0.00^{b}
AC04	0.41±0.14^{ab}	1.00±0.00^{b}
AC05	0.44±0.14^{ab}	1.00±0.00^{b}
AC06	0.41±0.19^{ab}	0.98±0.02^{ab}
AC07	0.26±0.04^{a}	1.00±0.00^{b}
AC08	0.23±0.02^{a}	0.97±0.03^{ab}
AC09	0.43±0.07^{ab}	1.00±0.00^{b}
AC10	0.67±0.07^{b}	1.00±0.00^{b}

Means with the same alphabet within a column are not significantly different from one another at $P \leq 0.05$ using Duncan Multiple Range Test (DMRT). Values are means of measurements ± Standard error (S.E). DSS7, 14, 21: Susceptibility to drought score at day 7, 14 and day 21; PPW7, 14, 21: Percentage of plants wilted at day 7, 14 and 21; LWI7, 21: Leaf wilting index at day 7 and 21.

Table 3: Recovery parameters after two weeks of re-watering of accessions of cowpea under drought stress at vegetative stage

ACCESSION	PREC (%)	STG	STR
AC01	31.11±4.44^{bc}	5.00±0.00^{b}	3.00±0.00^{b}
AC02	28.88±2.21^{bc}	4.33±0.67^{b}	2.33±0.67^{b}
AC03	12.20±1.10^{ab}	5.00±0.00^{b}	3.00±0.00^{b}
AC04	28.88±2.21^{bc}	5.00±0.00^{b}	3.00±0.00^{b}
AC05	44.44±2.22^{c}	5.00±0.00^{b}	3.00±0.00^{b}
AC06	77.78±11.11^{d}	5.00±0.00^{b}	3.00±0.00^{b}
AC07	35.55±11.12^{c}	5.00±0.00^{b}	3.00±0.00^{b}
AC08	0.00±0.00^{a}	1.00±0.00^{a}	0.00±0.00^{a}
AC09	35.55±8.88^{c}	5.00±0.00^{b}	3.00±0.00^{b}
AC10	0.00±0.00^{a}	1.00±0.00^{a}	0.00±0.00^{a}

Means with the same alphabet within a column are not significantly different from one another at $P \leq 0.05$ using Duncan Multiple Range Test (DMRT). Values are means of measurements ± Standard error (S.E). PREC: Percentage recovery; STG: Stem greenness; STR: Stem re-growth.

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Table 4: Effect of drought stress on length of terminal leaflet (cm) of accessions of cowpea at vegetative stage

ACCESSION	ITLL	TLL7	Reduction (%)
AC01	9.03±0.88^{cd}	8.42±0.95^{b}	6.76
AC02	9.26±0.61^{cd}	9.08±0.98^{b}	1.94
AC03	8.85±0.59^{bcd}	7.99±0.86^{ab}	9.72
AC04	9.53±0.43^{d}	9.09±0.42^{b}	4.62
AC05	8.71±0.69^{bcd}	8.92±0.57^{b}	-2.41
AC06	7.77±0.71^{abcd}	7.11±0.74^{ab}	8.49
AC07	6.49±0.24^{a}	6.02±0.24^{a}	7.24
AC08	9.19±0.49^{cd}	8.84±0.35^{b}	3.81
AC09	7.59±0.11^{abc}	8.19±0.23^{b}	-7.90
AC10	7.12±0.22^{ab}	7.12±0.01^{ab}	0.00

Means with the same alphabet within a column are not significantly different from one another at P ≤ 0.05 using Duncan Multiple Range Test (DMRT). Values are means of measurements ± Standard error (S.E). ITLL: Initial length of terminal leaflet; TLL 7: Length of terminal leaflet at day 7.

Table 5: Effect of drought stress on width of terminal leaflet (cm) of accessions of cowpea at vegetative stage

ACCESSION	ITLW	TLW7	Reduction (%)
AC01	4.67±0.48^{b}	4.16±0.58^{abcd}	10.92
AC02	5.30±0.51^{b}	4.91±0.56^{d}	7.36
AC03	4.23±0.66^{ab}	3.73±0.66^{abcd}	11.82
AC04	5.51±0.16^{b}	4.95±0.15^{d}	10.16
AC05	4.80±0.59^{b}	4.39±0.48^{bcd}	8.54
AC06	4.73±0.45^{b}	4.68±0.45^{cd}	1.06
AC07	3.19±0.21^{a}	2.89±0.19^{a}	9.40
AC08	3.30±0.09^{a}	2.92±0.02^{a}	11.52
AC09	3.42±0.08^{a}	3.48±0.23^{abc}	-1.75
AC10	3.41±0.04^{a}	3.30±0.00^{ab}	3.23

Means with the same alphabet within a column are not significantly different from one another at P ≤ 0.05 using Duncan Multiple Range Test (DMRT). Values are means of measurements ± Standard error (S.E). ITLW: Initial width of terminal leaflet; TLW 7: Width of terminal leaflet at day 7.

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Table 6: Effect of drought stress on plant height (cm) of accessions of cowpea at vegetative stage

ACCESSION	IPH	PH7	PH14	Increase (%)
AC01	22.50±2.89[ab]	27.58±5.31[ab]	27.58±5.31[ab]	22.58
AC02	27.12±1.68[ab]	31.10±3.05[ab]	31.10±3.05[ab]	14.68
AC03	25.93±0.28[ab]	26.80±0.03[ab]	26.80±0.03[ab]	3.36
AC04	24.57±1.23[ab]	25.92±0.99[ab]	25.92±0.99[ab]	5.49
AC05	28.28±2.86[b]	33.34±7.03[b]	33.34±7.03[b]	17.89
AC06	25.45±3.03[ab]	27.87±2.53[ab]	27.87±2.53[ab]	9.51
AC07	23.28±1.05[ab]	23.81±1.30[ab]	23.81±1.31[ab]	2.28
AC08	24.35±0.67[ab]	28.63±1.29[ab]	28.63±1.29[ab]	17.58
AC09	22.96±0.66[ab]	23.74±0.73[ab]	23.74±0.73[ab]	3.39
AC10	21.02±1.30[a]	21.09±1.38[a]	21.09±1.38[a]	0.33

Means with the same alphabet within a column are not significantly different from one another at P ≤ 0.05 using Duncan Multiple Range Test (DMRT). Values are means of measurements ± Standard error (S.E). IPH: Initial plant height; PH 7, 14: Plant height at day 7 and 14.

Table 7: Effect of drought stress on stem girth (mm) of accessions of cowpea at vegetative stage

ACCESSION	ISG	SG7	SG14	Reduction (%)
AC01	3.81±0.37[ab]	3.32±0.35[bc]	2.11±0.54[b]	44.62
AC02	3.80±0.37[ab]	3.64±0.48[d]	2.64±0.43[d]	30.53
AC03	3.32±0.11[a]	3.10±0.09[a]	2.22±0.30[bc]	33.13
AC04	4.07±0.11[ab]	3.52±0.13[c]	2.23±0.10[bc]	45.21
AC05	3.58±0.33[ab]	3.30±0.23[bc]	2.10±0.22[b]	41.34
AC06	3.71±0.29[ab]	3.25±0.16[a]	2.20±0.27[bc]	40.7
AC07	3.51±0.26[ab]	3.31±0.33[bc]	1.89±0.45[a]	46.15
AC08	4.24±0.14[b]	3.88±0.16[d]	2.55±0.11[d]	39.86
AC09	3.83±0.14[ab]	3.49±0.06[c]	2.32±0.13[c]	39.43
AC10	3.38±0.17[a]	3.29±0.19[b]	2.09±0.23[b]	38.17

Means with the same alphabet within a column are not significantly different from one another at P ≤ 0.05 using Duncan Multiple Range Test (DMRT). Values are means of measurements ± Standard error (S.E). ISG: Initial stem girth; SG 7 and 14: Stem girth at day 7, 14 and 21.

Table 8: Effect of drought stress on number of pods per plant (PDP) of accessions of cowpea at vegetative stage

ACCESSION	PDP
AC01	3.33±0.67[bc]
AC02	4.00±1.53[c]
AC03	0.00±0.00[a]
AC04	0.00±0.00[a]
AC05	1.00±0.00[ab]
AC06	0.00±0.00[a]
AC07	15.33±0.88[d]
AC08	0.00±0.00[a]
AC09	13.33±1.76[d]
AC10	0.00±0.00[a]

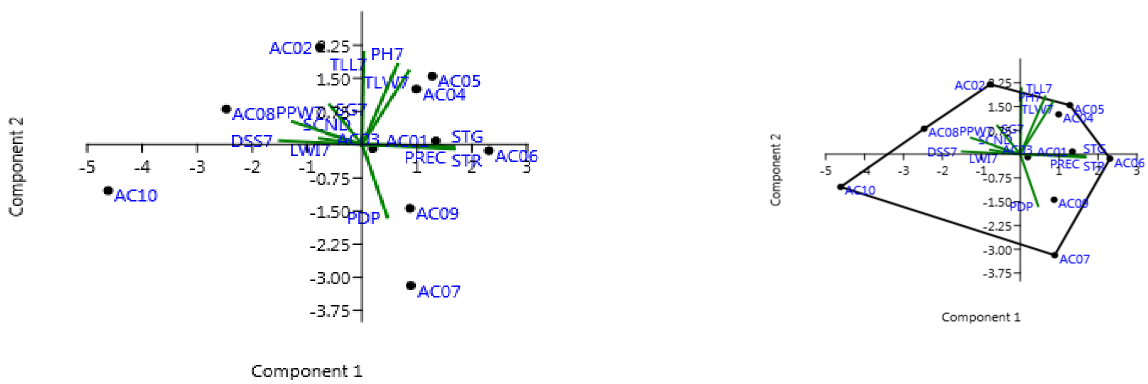
Means with the same alphabet within a column are not significantly different from one another at P ≤ 0.05 using Duncan Multiple Range Test (DMRT). Values are means of measurements ± Standard error (S.E).

ISSN: 0974 – 3987
IJBST (2020), 13(2): 18-33
DOI: http://doi.org/10.5281/zenodo.4019163

Table 9: Stomata conductance (mmol m^{-2}s^{-1}) of accessions of cowpea under drought stress at vegetative stage

ACCESSION	SCND (mmol m^{-2}s^{-1})	RANK
AC01	160.89 ± 49.72^{ab}	6
AC02	647.62 ± 40.57^{e}	1
AC03	400.92 ± 48.92^{d}	2
AC04	85.93 ± 10.81^{a}	7
AC05	68.73 ± 3.94^{a}	8
AC06	52.85 ± 5.90^{a}	9
AC07	334.18 ± 85.49^{cd}	3
AC08	177.53 ± 31.14^{ab}	5
AC09	89.38 ± 23.18^{a}	7
AC10	275.22 ± 2.53^{bc}	4

Means with the same alphabet within a column are not significantly different from one another at $P \leq 0.05$ using Duncan Multiple Range Test (DMRT). Values are means of measurements ± Standard error (S.E). SCND: Stomata conductance.



a

b

Figure 1: Bi-plots of Principal Component analysis of accessions of cowpea under drought stress at vegetative stage showing the interrelationships among different shoot traits at day 7 with recovery parameters, stomata conductance and number of pods (a) and polygon view of the genotype x trait bi-plot (b). DSS7: Susceptibility to drought score at day 7; PPW7: Percentage of plants wilted at day 7; LWI7: Leaf wilting index at day 7; SCND: Stomata conductance; PREC: Percentage recovery; STG: Stem greenness; STR: Stem re-growth; TLL7: Length of terminal leaflet at day 7; TLW7: Width of terminal leaflet at day 7; SG7: Stem girth at day 7; PDP: Number of pods per plant.

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ISSN: 0974 – 3987
IJBST (2020), 13(2): 18-33

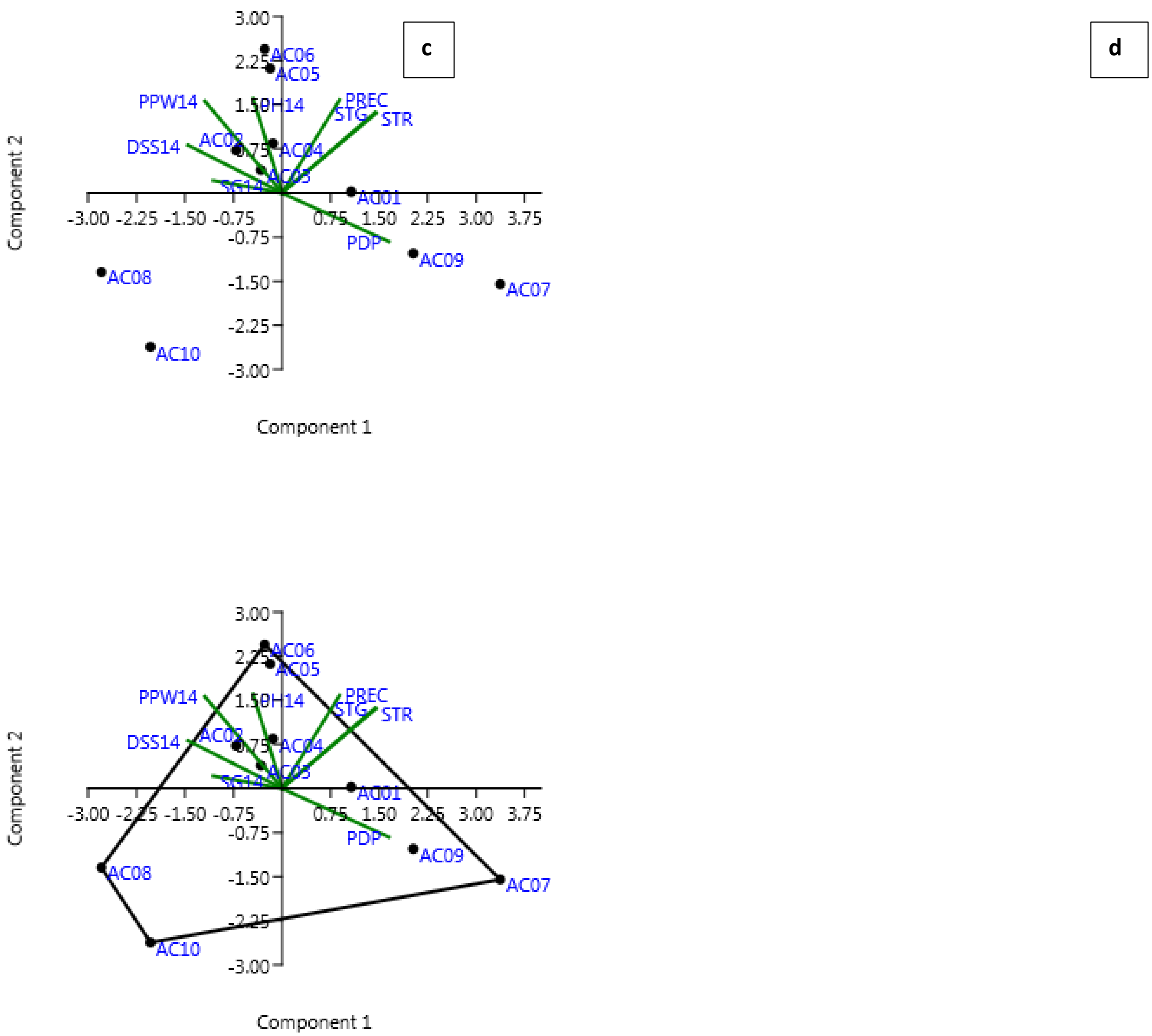

Figure 1 cont'd: Bi-plots of Principal Component analysis of accessions of cowpea under drought stress at vegetative stage showing the interrelationships among different shoot traits at day 14 with recovery parameters, stomata conductance and number of pods (c) and polygon view of the genotype x trait bi-plot (d). DSS14: Susceptibility to drought score at day 14; PPW14: Percentage of plants wilted at day 14; SCND: Stomata conductance; PREC: Percentage recovery; STG: Stem greenness; STR: Stem re-growth; TLL14: Length of terminal leaflet at day 14; TLW14: Width of terminal leaflet at day 14; SG14: Stem girth at day 14; PDP: Number of pods per plant.

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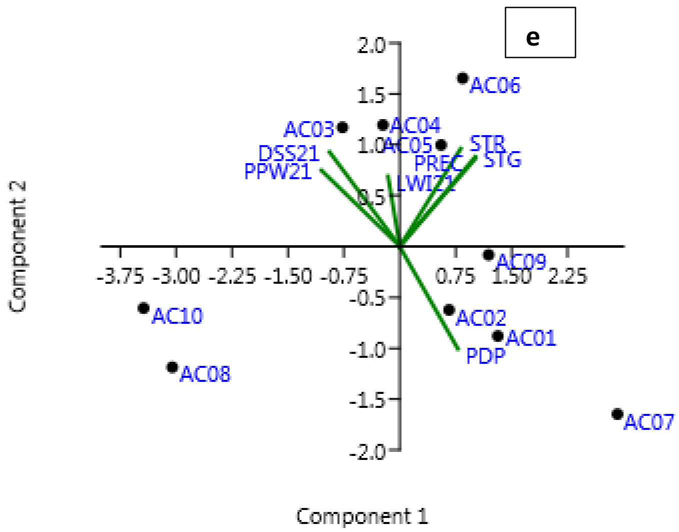
IJBST (2020), 13(2): 18-33



f

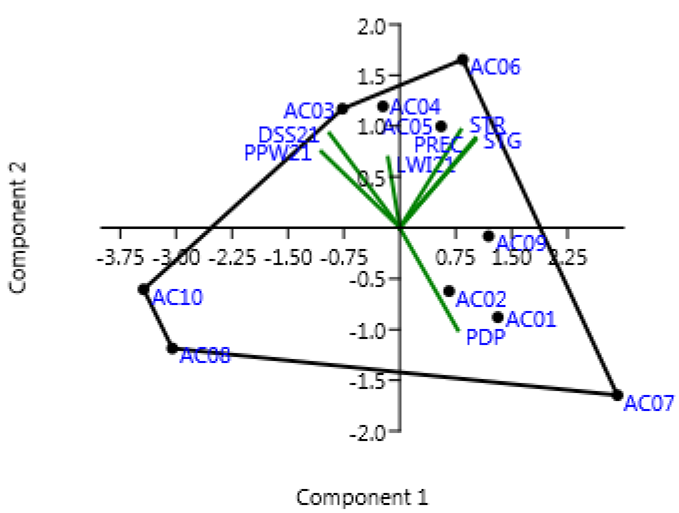


Figure 1 cont'd: Bi-plots of Principal Component analysis (PCA) of accessions of cowpea under drought stress at vegetative stage showing the interrelationships among different shoot traits at day 21 with recovery parameters, stomata conductance and number of pods (e) and polygon view of the genotype x trait bi-plot (f). DSS21: Susceptibility to drought score at day 21; PPW21: Percentage of plants wilted at day 21; SCND: Stomata conductance; PREC: Percentage recovery; STG: Stem greenness; STR: Stem re-growth; PDP: Number of pods per plant.

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CONCLUSION

According to biplot analysis, wilting traits, recovery parameters and number of pods per plant were the best traits that can be used for screening cowpea for tolerance at vegetative stage. The most susceptible accessions included AC08, AC10 and AC03, while the most tolerant accession based on recovery was AC06 and the most tolerant based on number of pods was AC07. This was consistent with the outcome of the screening done at the seedling stage which suggest that screening for drought tolerance at any developmental stage of cowpea will be reliable in breeding tolerant lines. Crosses involving AC07 and AC06 would mostly yield highly tolerant accessions with high capability to produce pods under drought conditions and maintain tissue moisture. The most susceptible accessions can be improved by crossing them with AC07 and AC06. AC09, AC05, AC01 and AC02 were moderately tolerant, while AC04 was moderately susceptible.

Research / Educational / Industrial / Other Reference Feasibility

The manuscript will help in future breeding program of cowpea for drought tolerance. It will serve as a reference that academic, research and other institutions can use in the breeding program of cowpea.

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